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In-season leaf damage by a biocontrol agent explains reproductive output of an invasive plant species

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Abstract

One of the biggest challenges in classical biological control of invasive weeds is predicting the likelihood of success. *Ambrosia artemisiifolia*, a North American plant species that has become invasive in Europe, causes economic losses due to health problems resulting from its huge amount of highly allergenic pollen and as a weed to agricultural crops resulting from high seed densities. Here we assessed whether the pollen and seed output of the annual *A. artemisiifolia* (at the end of the season) is related to in-season abundance of, or damage by, the accidentally introduced biological control agent *Ophraella communa*. We monitored the growth and leaf damage of individually labelled *A. artemisiifolia* plants at four locations in Northern Italy and recorded abundance of different *O. communa* life stages at regular intervals. We found that the in-season level of leaf damage by *O. communa* consistently helped to explain seed production in combination with plant volume and site throughout the season. Feeding damage, plant volume and site also explained pollen production by *A. artemisiifolia* six weeks before male flower formation. At three out of four sites, plants with more than 10% leaf damage in mid-June or early July had a very low likelihood of seed formation. Leaf damage proved to be a better explanatory variable than *O. communa* abundance.

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Our results suggest that the monitoring of the in-season leaf damage can help to project the local impact of *O. communa* on *A. artemisiifolia* at the end of the season and thus inform management regarding the needs for additional measures to control this prominent invader.

Keywords

Ambrosia artemisiifolia, biological invasions, classical biological control, common ragweed, herbivory, *Ophraella communa*

Introduction

It is now well established that plant species that are introduced into areas outside their native range and become invasive can wreak serious impact on nature and human well-being (Vilà et al. 2011). One option to mitigate the negative impacts of invasive alien species is classical biological control, i.e. the use of specialist natural enemies from the native range to reduce invader densities below an economic and ecological threshold or to slow down their spread (Müller-Schärer and Schaffner 2008). Classical biological control of invasive alien plant species has been implemented worldwide for more than 120 years (Winston et al. 2014). The method is used either alone or in combination with other weed management practices (Müller-Schärer and Collins 2012, Lake and Minteer 2018).

One of the biggest challenges in classical biological control of weeds is predicting the likelihood of success, and thus the necessity for considering additional management practices (Schwarzländer et al. 2018). Once established, biological control agents need to reach high densities to impact the target plant to such an extent that vital rates, and ultimately its population growth rate, are negatively affected (Jamieson et al. 2012). In cases of successful biological control of invasive alien plant species, population densities of the introduced biological control agents often reach numbers several orders higher than observed within their native range (Müller-Schärer and Schaffner 2008). However, whether biological control agents are able to build up high densities depends, among others, on the weather and climate conditions they encounter in the new range (Weed and Schwarzländer 2014; Mills 2018). Including biological control in integrated weed management presupposes an understanding of the likely abundance of the biological control agent and its impact on the target weed in a given region or year.

Predicting the impact of herbivore abundance on plants has a long history in crop pest forecasting (Magarey and Isard 2017), where it is used to support decision making regarding the scheduling of pest management interventions. For example, Lemic et al. (2016) found a strong positive correlation between the number of noctuid moths caught in pheromone traps and damage to sugar beet crops, which can be used to inform the timing of insecticide application to keep damage under an economical threshold level. This principle is also used, among many others, in the management of codling moth in apple orchards (Rather et al. 2018), of western corn rootworm in

maize (Kos et al. 2014) and of navel orangeworm in almond plantations (Rosenheim et al. 2017). Thus, economic threshold levels are used in pest predictive models to decide when to apply pesticides. Along the same lines, a threshold level could be used in biological control programmes to inform weed managers whether or not additional control measures are required to achieve pre-defined management objectives. While the use of threshold levels in integrated weed management seems intuitive, there are only a few examples in classical biological control of weeds where in-season parameters related to herbivore abundance or damage have been identified that would allow informed management decisions. For example, Ding et al. (2006) estimated the number of larvae of the leaf beetle *Galerucella birmanica* necessary to control growth and reproduction of water chestnut, an invasive weed in several continents. Similarly, Häfliger et al. (2006) showed a negative linear relationship between attack rates of the stem boring noctuid *Archanara geminipuncta* early in the season and the performance of the invasive reed *Phragmites australis* towards the end of the season.

Ambrosia artemisiifolia L. (*Asteraceae*) is one of the most notorious plant invaders in Europe (Essl et al. 2015, Müller-Schärer et al. 2018). Originating from North America, it has been considered a noxious weed in Europe since the early 1920s (Csontos et al. 2010). In some parts of the invaded range, it causes serious yield losses in spring-sown crops such as maize, soy bean, and sunflower (e.g. Kőmives et al. 2006). Even more importantly, it produces large amounts of highly allergenic pollen annually, which causes substantial medical costs and reduced quality of life among the allergic population (Smith et al. 2013, Mouttet et al. 2018, Schaffner et al. in press). Current management tools for *A. artemisiifolia* in Europe comprise mechanical and chemical control, which are applied in agricultural environments and along roadsides (Milakovic and Karrer 2016, Lommen et al. 2018c). Due to their costs, impracticality, and/or their negative impact on the natural environment, these tools are unsuitable for most other habitat types where common ragweed thrives, such as wasteland, riversides, or on small patches of ruderal land. As both the spread and health impact of common ragweed in Europe are likely to increase with the changing climate (Storkey et al. 2014, Hamaoui-Laguel et al. 2015, Sun et al. 2017), and the number of persons sensitised to *A. artemisiifolia* pollen is rising (Lake et al. 2016), alternative methods such as biological control will be important to help to control this invasive weed (Gerber et al. 2011). Biological control of *A. artemisiifolia* has already been implemented in Australia and China (Palmer et al. 2010, Zhou et al. 2014). In China, biological control of common ragweed is based on joint mass releases of the deliberately introduced noctuid moth *Epiblema strenuana* Walker and the accidentally introduced leaf beetle *Ophraella communa* LeSage (Zhou et al. 2014).

In Europe, *O. communa* was found for the first time in Northern Italy in 2013, probably also due to an accidental introduction (Bosio et al. 2014, Müller-Schärer et al. 2014). Since then, the beetle has become widely established in Northern Italy and has been spreading all over the Po Plain (Augustinus et al. 2015, Lommen et al. 2017b). In its current range in Europe, the beetle can complete up to four generations per year, which can result in complete defoliation and impaired reproduction of *A. artemisiifolia*

plants (Bosio et al. 2014, Müller-Schärer et al. 2014). Since the first sighting of high abundances of *O. communa* in Northern-Italy in 2013, yearly airborne ragweed pollen counts in the region have decreased by 80%. As this drop in airborne pollen counts could not be explained by land use change or meteorological factors, it is most likely attributable to feeding damage by *O. communa* (Bonini et al. 2015a, Bonini et al. 2015b). At the field plot level, Lommen et al (2018b) showed a negative effect of the presence of *O. communa* on the density of *A. artemisiifolia* seeds produced. However, the observed effect of *O. communa* on *A. artemisiifolia* plants varied considerably, both at the spatial and temporal scale (Lommen and Augustinus, unpublished data).

In Northern Italy, the yearly peak of *O. communa* population size is only reached at the time when the first flower buds are produced. Identifying earlier, in-season indicators that are related to the level of biological control at the end of the season could help to project whether in a particular season or location *O. communa* damages *A. artemisiifolia* to such an extent that it prevents plants from reproduction, i.e. from producing pollen (which impacts human health) or seeds (which impacts long-term population dynamics and crop yield).

Here we report on a field experiment to assess whether abundance of or damage by *O. communa* during the season is related to *A. artemisiifolia* reproduction at the end of the season. We followed individually labelled *A. artemisiifolia* plants in four locations in Northern Italy during the summer of 2016 to answer the following questions: (1) what is the in-season variation in a) in-season survival of *A. artemisiifolia*, b) the number of *O. communa* individuals of, and leaf damage caused by *O. communa* on individual *A. artemisiifolia* plants , and (2) what is the effect of in-season *O. communa* abundance or leaf damage on *A. artemisiifolia* reproduction at the end of the season?

Material and methods

Study species

Ambrosia artemisiifolia is an annual plant that has invaded areas in all continents except Antarctica (Csontos et al. 2010, Essl 2015). The monoecious plant produces racemes with male flower heads that release highly allergenic pollen. The wind-pollinated female flowers are located in clusters in the leaf axils, with each flower producing one single seed. The seeds can remain viable in the soil for up to 40 years (Toole and Brown 1946). In Northern Italy, the first plants emerge in early April but they can emerge anytime until late summer, e.g. after soil disturbance or heavy rains. They form male flowers by mid-August, producing pollen in August and September (Bonini et al. 2015a), while female flowers are formed in early September, and produce seeds from mid- to late September (Fogliatto et al. 2019, Lommen et al. 2018b).

Ophraella communa is a multivoltine leaf beetle which overwinters at the adult stage and lays eggs in egg batches in spring. The beetle then goes through three larval stages, which feed on the green parts of the host plant. It then pupates and starts mating

shortly after emergence from the lightly woven cocoon. Adults feed on green parts of the plant as well. In Northern Italy, the beetle can complete up to 4 generations per year (Mouttet et al. 2018). In southern China, where climatic conditions appear to be particularly suitable, *O. communa* concludes five generations in a year (Meng et al. 2007).

Study sites

We selected three former crop fields and one meadow with natural populations of both *A. artemisiifolia* and *O. communa* in the Po Plain of the Italian Piedmont and Lombardy regions (see Suppl. material 1). The sites were in different successional stages, thus allowing us to include variation in interspecific competition and ragweed growth rate, which likely affect biocontrol impact. One field site (Magnago) had been converted from woodland to grassland 4 years prior to the experiment. Another site (Unito) was a former crop site that had not been used for agriculture for the past two years and was dominated by grasses. Two other crop sites (Magenta, Busto Arsizio) were in agricultural use until very recently and were still dominated by early-succession weeds. The Busto Arsizio site was tilled and prepared for maize production (as grown adjacent) by a local farmer two weeks before commencing the experiment. The preparations included application of fertilizer, but no application of pre-emergence herbicides. The size of the study plots in each site ranged from 125–400 m^2. To ensure that enough plants were available for the experiment, we disturbed the plots in April by mowing and raking to encourage recruitment of *A. artemisiifolia* from the soil seedbank.

Selection of plants

The study plants were selected between 13 and 18 June 2016, when *A. artemisiifolia* was between the 4- and the 12-leaf stage. We maximised the variation in initial size of *A. artemisiifolia* by randomly measuring plants at each site for 10 minutes and separating them into three equally numbered size classes (small, medium, large). We then laid transects of 20 m length through the study plots and selected 20 plants per size class along this transect, with an as homogeneous distribution over the site as possible. Minimum distance between selected plants was 50 cm and the maximum distance away from the transect was 2 m. Plants were individually marked with an aluminium label around the stem and a bamboo stick.

Timing of the study

We decided to start our experiment in mid-June to exclude background seedling mortality from the dataset, since seedling establishment can vary considerably within and among sites (Rothrock et al. 1993). Furthermore, *A. artemisiifolia* has a long germina-

tion period (Kazinczi et al. 2008) and possesses high variability in germination rate and onset even among individuals of a population (Fogliatto et al. 2019). Thus, starting our experiment in mid-June allowed us to include plants that had germinated later and thus to cover a larger range in plant size.

Insecticide treatment

In order to increase intra-site variation in abundance of and damage by *O. communa*, two subplots of approximately 5 m long along the transect were selected at random for insecticide application. The two subplots contained in total 12 labelled plants (4 plants per size class) per site. These subplots were sprayed twice a month with insecticides, alternating between contact and systemic insecticides. We used Lambda-Cyhalothrin in a dosage of 20g/ha (Syngenta KarateZeon) as contact insecticide, and a combination of Acetamiprid in a dosage of 100g/ha (Sipkam EPIK), and Deltamethrin in a dosage of 20g/ha (Bayer DecisEVO) as systemic insecticides. Insecticides were applied at a spray volume of 1000L/ha using a backpack sprayer. Previous studies revealed that there is no direct effect of this insecticide treatment on the measured plant parameters (Lommen et al. 2018a). The rest of the study plots were sprayed with the equivalent amount of water with the same intervals.

Measurements

Plant survival and size, *O. communa* abundance and leaf damage caused by *O. communa* were assessed on individual plants six times ("censuses") at three-week intervals from mid-June until mid-September 2016 (see exact dates in Suppl. material 2). To assess plant survival, plants were scored as 'alive' as long as parts of their above-ground biomass were green; for example, completely defoliated plants were scored alive when parts of the main stem or lateral shoots were still green. To monitor plant size, we measured height (from the ground to the highest point when the plant was held erect) and width (at the widest span) of the plants and used these values to calculate volume, using the following formula:

$$Volume = height * \pi * (width/4)^2$$

To assess the abundance of *O. communa* on individual plants, we counted the number of *O. communa* egg batches, larvae >5 mm long (larger L2 and L3 larvae), and the number of adults on each labelled plant at each census. We disregarded egg batches with less than 5 eggs, because laboratory experiments indicated that eggs from small egg batches are mostly unfertilised (Augustinus, unpublished data). As small larvae are difficult to find since they can hide in buds and flowers, we did not count these to minimize observer errors. In addition, we measured leaf damage per plant by estimat-

ing the percent leaf area removed by *O. communa* from the total leaf area if the plant was intact (plants without leaves were given a value of 100% area removed). We did not score damage that was clearly not caused by *O. communa* (e.g. with traces of snail mucus). However, we never observed other leaf-chewing insect herbivores on *A. artemisiifolia* than *O. communa*, and rarely found traces of molluscs.

To estimate levels of plant competition early in the season, we assessed percent bare soil in a 50×50cm square around each marked plant in early July. A square frame of 50×50cm was laid around a plant and the fraction of that surface covered by bare soil, when projecting the vegetation onto the ground, was estimated by at least two persons and the average taken. Stones or dead leaf material were scored as bare soil as well. In late August, we measured the summed length of all racemes per plant as a proxy for pollen production (Lommen et al. 2018b). Seed formation was assessed between 19 and 25 September 2016 by counting the number of seeds and female flowers (each flower gives rise to a single seed) produced per plant before seed rain.

Statistical analysis

To compare the change in leaf damage over time between sites, we conducted a repeated measures ANOVA with damage as response variable, site as fixed variable, and census as random effect. The fit of the residuals was evaluated graphically, and we took the square root of damage to obtain a better fit.

Because of the highly zero-inflated nature of our data, we applied a hurdle approach to analyse the effect of *O. communa* numbers on male (i.e. pollen) and female (i.e. seeds) *A. artemisiifolia* reproduction by first using presence/absence of racemes (pollen-bearing structures) in late August, and of seeds in mid-September to assess the probability of male and female reproduction, respectively. In a second step, we analysed the quantity of male and female reproduction conditional on the probability of reproduction (i.e. only using plants that did produce), using raceme length (as a proxy for the number of pollen produced), and numbers of seeds as response variables, respectively.

In the first part of the hurdle approach, we assessed the effect of *O. communa* on likelihood of raceme or seed formation in separate analyses by formulating generalised linear models for each of the first four (for raceme formation) or five (for seed formation) censuses. As fixed effects we included site, the natural logarithm of plant volume, as well as none or one of the four *O. communa*-related variables (number of eggs per plant, number of pupae per plant, number of adults per plant, and percent leaf damage) in each model, as these were inter-correlated. We produced models with and without an interaction term for the *O. communa*-related variable and plant volume, and with and without percent bare soil. We compared all resulting 18 models for each response variable at each census and selected the model with the lowest conditional Akaike information criterion (AICc) value, which penalizes models with more param-

eters (Burnham and Anderson 2002). To assess the fit of each model, we added Nagelkerke's pseudo r-squared (Tables 1, 2). We displayed the effect size of the explanatory variables by plotting the odds ratios for all explanatory variables of the best performing model per census (Figures 4, 5).

In the second step of the hurdle approach, we assessed the effect of *O. communa* on total raceme length or number of seeds of those plants that did produce racemes or seeds, respectively. We formulated a set of linear models for the natural logarithm of raceme length and number of seeds, assuming a Gaussian distribution of the response variable. We chose to use a Gaussian distribution over a Poisson distribution since it reduced AICc values of the fitted models by more than 5000 for every case. As fixed effects we included the natural logarithm of volume and site and added none or one of the four *O. communa*-related variables. To prevent overparameterization, we did not include bare soil and interactions with plant volume in these models, since the sample size of plants that successfully formed racemes and seeds was too low to include more than three fixed effects.

All analyses were conducted in R version 3.5.1 (2018–07–02) --"Feather Spray" (2018). Data were prepared using the readxl (Wickham and Bryan 2016) and reshape (Wickham 2007) packages, models were formulated in lme4 (Bates et al. 2015), model fits explored in DescTools (Signorell 2017) and MuMIn (Bartoń 2013), comparisons of damage levels between sites were conducted in agricolae (De Mendiburu 2019) and figures produced in ggplot2 (Wickham 2009) , sjPlot (Lüdecke 2018) and gridExtra (Auguie 2016) packages.

Results

Ophraella communa abundance and damage

Until late July, we found less than one egg batch, larva or adult of *O. communa* per plant (Fig. 1). The number of egg batches increased in early August but declined again in late August; after that, we found no more egg batches. Larvae and adult counts peaked in late August, with larval counts averaging 5 individuals per plant in late August (Fig. 1).

Plant volume steadily increased until late August, and decreased or stayed stable thereafter (Suppl. material 4). The few plants with no leaf damage during the course of the experiment were exclusively plants treated with insecticides (Fig. 2). Plants that were not treated with insecticides showed a moderate but continuous increase in percent leaf damage by *O. communa* until early August, reaching an average of approximately 20–50% leaf damage across all sites (Fig. 2). Between early and late August, the percent of leaf tissue damaged rapidly increased to approximately 85%. Plant mortality occurred throughout the experiment, but was most severe between late August and the beginning of September (Fig. 3), before natural mortality due to senescence occurred. Percent leaf damage varied significantly among sites (repeated measures ANOVA,

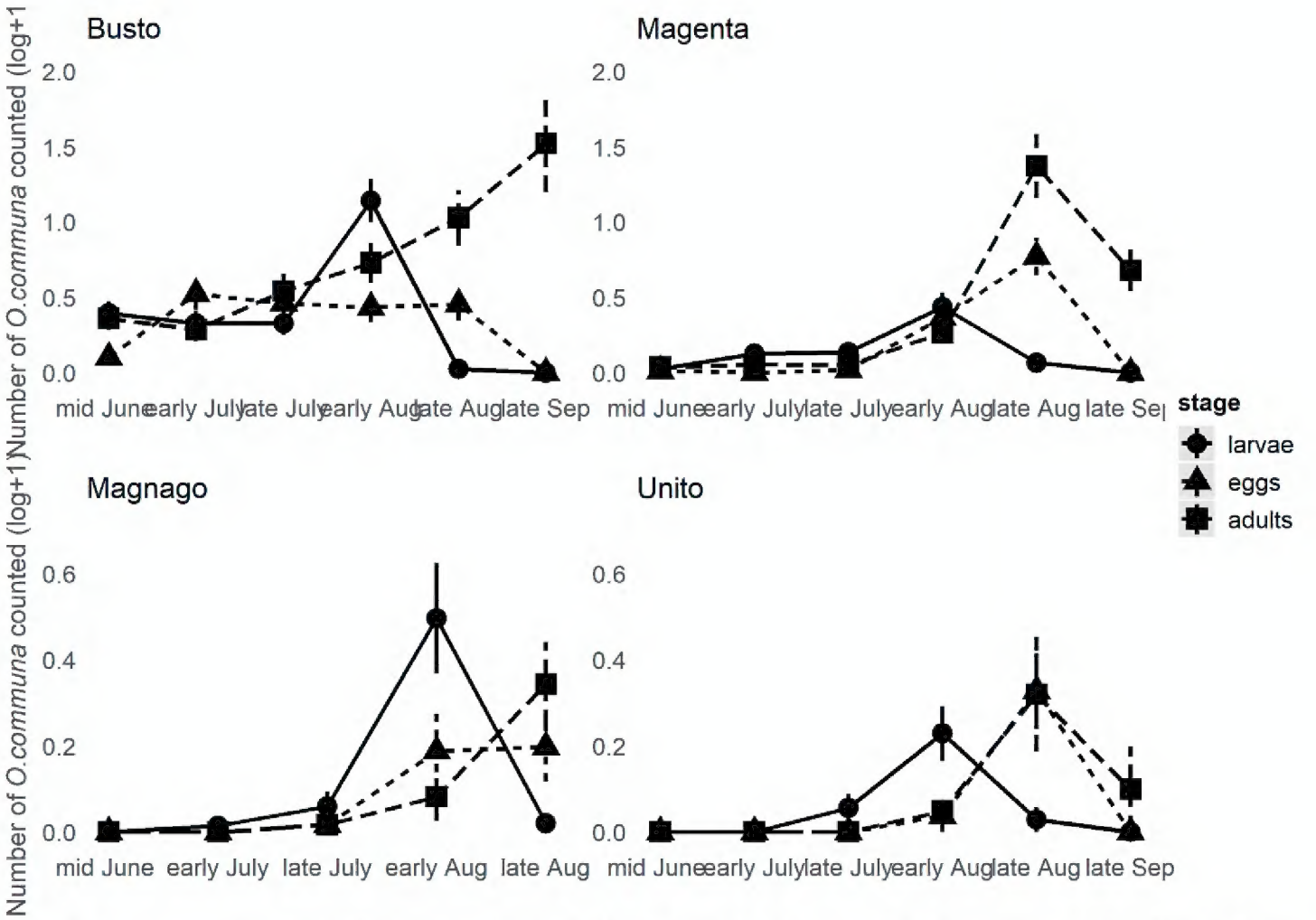

Figure 1. Average number of *O. communa* individuals per plant during the experiment in the four different sites. Different life stages are marked with different lines and symbols. Vertical lines indicate the standard error.

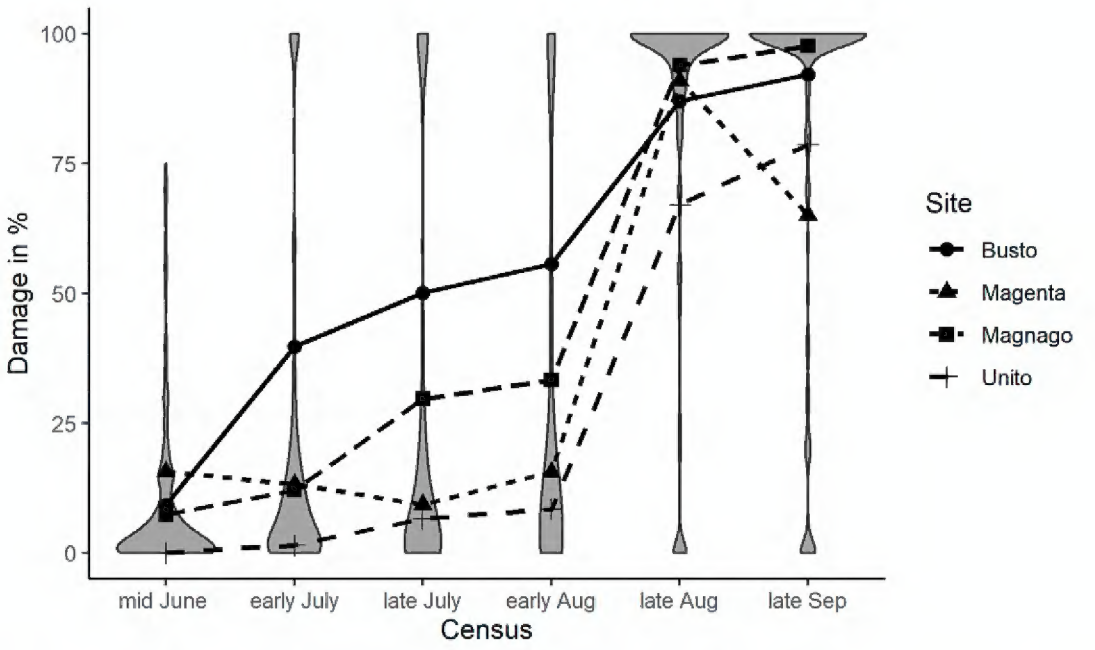

Figure 2. Violin plot of *Ambrosia artemisiifolia* leaf damage by *O. communa* feeding. The lines indicate the mean of the leaf damage scored on living plants in the different sites. The distribution of the damage measurements is shown with the grey shapes. Only damage of plants which were not treated with insecticides are displayed.

H = 147.33, df = 3, $p < 0.01$) , with Busto Arsizio exhibiting the highest percent leaf damage until early August and Unito the lowest percent leaf damage throughout the whole study period (Fig.2).

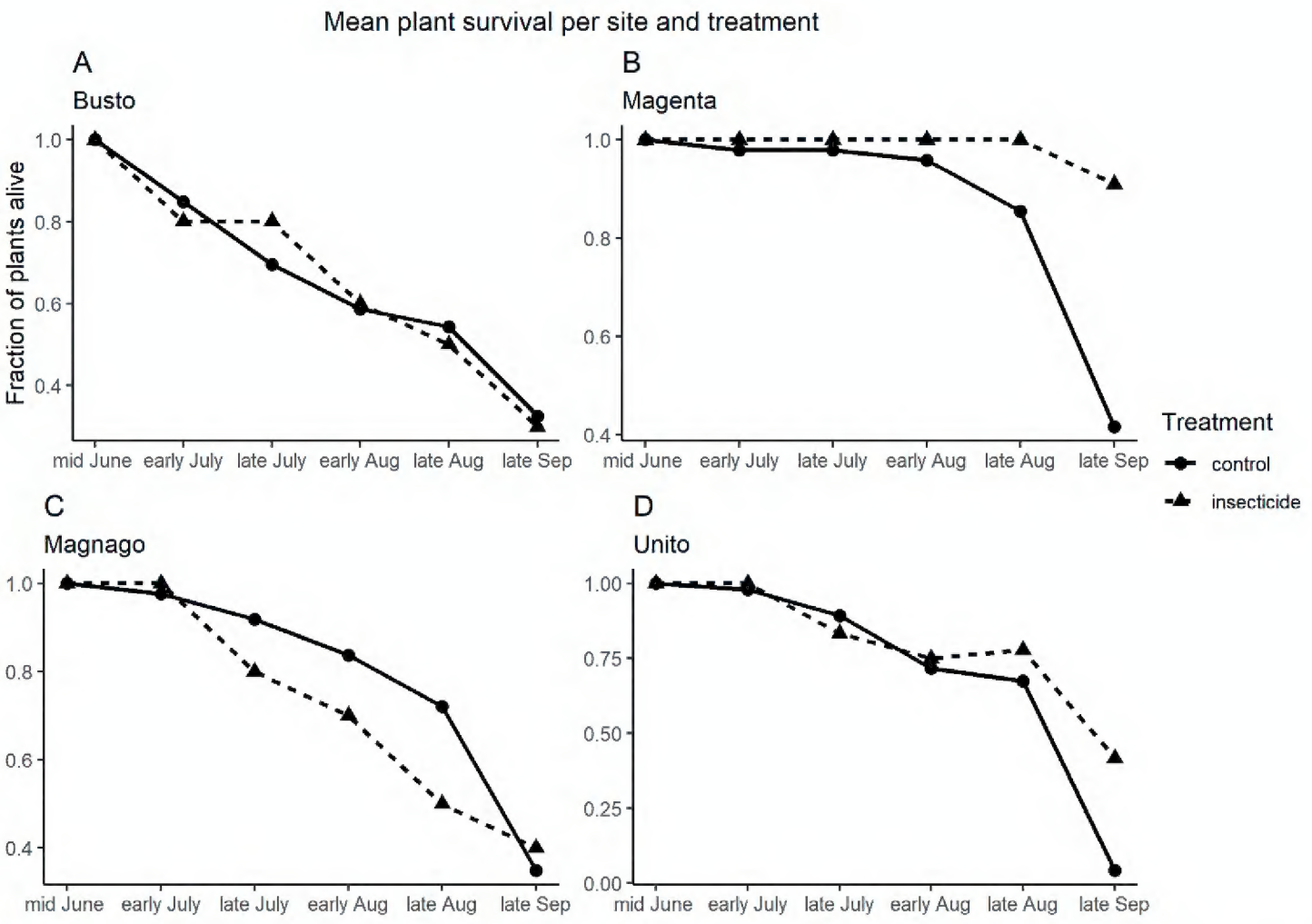

Figure 3. Fraction of plants exposed to *O. communa* and those treated with insecticides alive over time during the experiment.

Effect of *O. communa* on *A. artemisiifolia* reproduction

Models with the lowest delta AICc values (compared to the best performing model) for successful raceme formation included *O. communa* abundance parameters measured in early August (number of adults), and models with the lowest delta AICc values for raceme length of the plants that successfully formed racemes included *O. communa* abundance parameters measured in late July (number of egg batches) and early August (number of adults; see Table 1).

In the model with the lowest delta AICc value for successful raceme formation in late July, we found a positive relationship between leaf damage in percent and successful raceme formation (Fig. 4). In the models for raceme length, the number of egg batches per plant in late July was positively related to raceme length, and number of adults per plant was positively related to raceme length (Table 1).

The selected models for successful seed formation included *O. communa* abundance parameters measured in early July (number of adults per plant), early August (number of adults, larvae and egg batches per plant) and late August (number of adults and larvae per plant) (Table 2). Successful seed formation could best be explained by models including the number of adults per plant in early July, the number of adults, number of larvae or number of eggs per plant in early August, and the number of adults or larvae per plant in late August. In all cases, the number of *O. communa* had a negative effect on successful seed formation. Five out of six models had a lower AICc

Table 1. Delta AICc values (upper number) compared to the model with the lowest AICc value, pseudo r-squared (second number), odds ratio for the *O. communa* related factor (third number), and confidence interval of the odds ratio for the *O. communa* related factor (lowest number) for models showing correlation between chance of successful raceme formation (left part of table) and total raceme length of raceme-producing plants (right part of table) and explanatory factors at different censuses. Models, where the confidence interval of the odds ratio for the effect size of the *O. communa* related factor does not cross 0, are shaded. Models including interactions with volume and the explanatory factor are marked with '*'. Corresponding p-values can be found in Suppl. material 3.

Factor	Probability of raceme formation dependent on factor				Raceme length dependent on factor …			
	Mid-June	Early July	Late July	Early August	Mid-June	Early July	Late July	Early August
No *O. communa* parameter	59	58	46	6.2	13	16	8.1	1.5
	0.40	0.40	0.37	0.40	0.724	0.737	0.738	0.713
# egg batches	59	60	48	8.2	15	18	3.5	1.5
	0.41	0.40	0.37	0.40	0.72	0.74	0.74	0.72
	0.74	0.88	0.92	1.03	1.11	1.15	1.55	1.06
	(0.48, 1.15)	(0.58, 1.34)	(0.52,1.65)	(0.88, 1.22)	(0.70, 1.75)	(0.86, 1.55)	(1.12, 2.13)	(0.98, 1.16)
# larvae	61	60	47	7.8	14	18	10	2.7
	0.40	0.40	0.37	0.40	0.73	0.74	0.74	0.72
	0.82	1.01	0.74	1.13	1.82	1.08	1.20	1.06
	(0.25, 2.72)	(0.78, 1.32)	(0.42, 1.30)	(0.81, 1.57)	(0.70, 4.73)	(0.91, 1.28)	(0.76, 1.91)	(0.95, 1.19)
# adults	60	60	48	4.5*	15	16	10	**0**
	0.41	0.40	0.37	0.43	0.73	0.75	0.75	0.73
	0.85	1.05	0.95	24.61	1.13	1.25	1.09	1.13
	(0.58, 1.25)	(0.65, 1.70)	(0.73, 1.23)	(0.97, 624.27)	(0.72, 1.77)	(0.93, 1.67)	(0.91, 1.30)	(1.00, 1.28)
% leaf damage	59	58	39*	**0***	15	18	11	2.6
	0.41	0.41	0.43	0.46	0.73	0.74	0.74	0.72
	0.97	0.98	1.02	1.06	1.01	0.99	1.01	0.98
	(0.94, 1.01)	(0.96, 1.01)	(1.00, 1.04)	(0.96, 1.17)	(0.97, 1.04)	(0.97, 1.02)	(0.98, 1.03)	(0.95, 1.01)

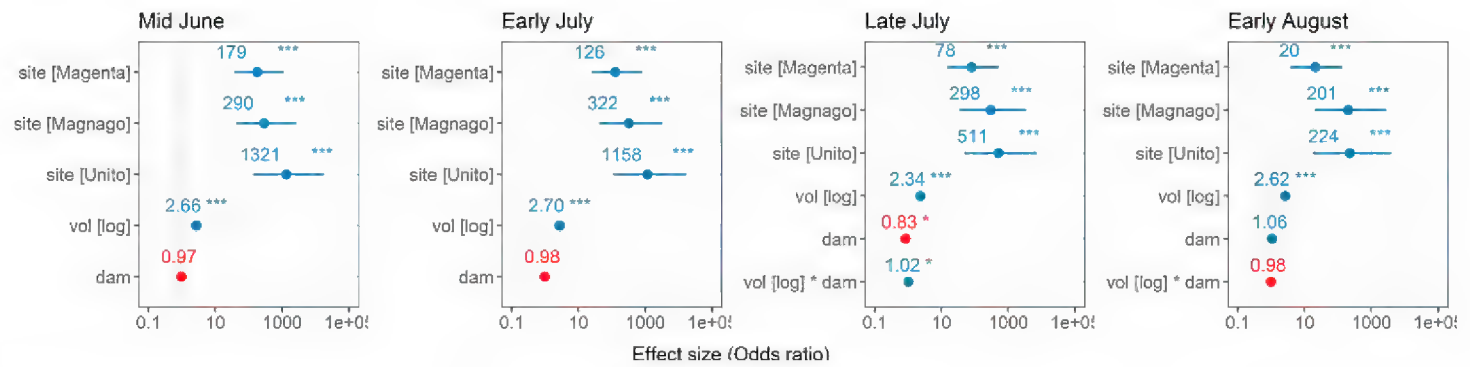

Figure 4. Odds-ratios of effect size of explanatory variables of the models with the lowest AIC per census, explaining successful raceme formation. Red dots/values <1 indicate that the effect is negative, blue dots/values >1 indicate that the effect is positive. The factor "site" with the corresponding site name in square brackets show the effect size of site compared to Busto. Plant volume "vol" (in cm^3) is log-transformed for the analysis, leaf damage in percent is abbreviated with "dam". In models with interaction between leaf damage in percent and volume, the effect size of this factor is described as "vol[log]*dam".

when an interaction term with volume and the number of *O. communa* individuals was included (see Table 2, Figure 5). Including percent bare soil did not increase any model fit. In contrast to the models for successful seed formation, none of the models

Table 2. Delta AICc values (upper number) compared to the model with the lowest AICc value, pseudo r-squared (second number), odds ratio for the *O. communa* related factor (third number), and confidence interval of the odds ratio for the *O. communa* related factor (lowest number) for models showing correlation between chance of successful seed formation (left part of table) and total seeds produced (right part of table) and explanatory factors at different censuses. Models, where the confidence interval of the odds ratio for the effect size of the *O. communa* related factor does not cross 0, are shaded. Models including interactions with volume and the explanatory factor are marked with '*'. Corresponding p-values can be found in Suppl. material 3.

Factor	Probability of seed formation dependent on…					Number of seeds produced dependent on…				
	Mid-June	Early July	Late July	Early August	Late August	Mid-June	Early July	Late July	Early August	Late August
No *O. communa* parameter	69	70	63	63	51	9.0	9.0	10	1.2	9.5
	0.16	0.17	0.16	0.21	0.16	0.85	0.86	0.85	0.86	0.85
# egg batches	66*	66*	61	28*	51*	9.5	12	13	1.1	13
	0.21	0.23	0.16	0.32	0.19	0.85	0.86	0.85	0.85	0.85
	1.79	0.00	1.30	0.03	0	1.19	1.06	1.16	0.91	1.04
	(0.83, 3.87)	(0, 4.51)	(0.76, 2.23)	(0.00, 0.44)	(0, inf)	(0.95, 1.49)	(0.85, 1.33)	(0.77, 1.73)	(0.82, 1.01)	(0.15, 6.99)
# larvae	60*	71	57	28*	26*	12	11	13	4.4	13
	0.26	0.18	0.17	0.32	0.40	0.84	0.86	0.85	0.83	0.85
	0.00	1.17	1.31	0.00	0.00	1.08	1.08	1.05	1.07	1.00
	(0, inf)	(0.90, 1.54)	(0.79, 2.18)	(0, 0.79)	(0, 0.27)	(0.54, 2.16)	(0.92, 1.25)	(0.71, 1.55)	(0.68, 1.69)	(0.77, 1.30)
# adults	68*	64	58	28*	31*	12	11	10	3.9	12
	0.19	0.23	0.17	0.31	0.36	0.84	0.86	0.87	0.84	0.85
	0.20	2.74	1.22	0	0.14	0.95	1.15	1.12	1.04	1.02
	(0, 109.11)	(1.15, 6.53)	(0.90, 1.65)	(0, 0.58)	(0.03, 0.74)	(0.75, 1.20)	(0.91, 1.45)	(0.97, 1.30)	(0.93, 1.16)	(0.95, 1.09)
% leaf damage	56*	59*	48*	1.9*	**0**	12	6.8	12	3.2	**0**
	0.29	0.27	0.30	0.51	0.57	0.83	0.88	0.86	0.84	0.90
	0.40	0.68	0.50	0.06	0.93	1.02	0.98	0.99	0.96	0.98
	(0.19, 0.83)	(0.51, 0.92)	(0.31, 0.80)	(0.01, 0.35)	(0.89, 0.98)	(0.87, 1.19)	(0.96, 0.99)	(0.97, 1.01)	(0.88, 1.04)	(0.97, 0.99)

with the lowest AICc values for number of seeds produced by successfully reproducing plants included an *O. communa* abundance parameter (Table 2).

Effect of percent leaf damage by *O. communa* on *A. artemisiifolia* reproduction

Including percent leaf damage by *O. communa* in models for successful raceme formation generated the models with the lowest AICc values for late July and early August, and including percent leaf damage by *O. communa* measured in late July generated the respective model with the lowest AICc value for raceme length of plants that successfully formed racemes (see Table 1). In those cases where damage reduced the probability of raceme formation, an interaction term with volume increased the fit of the models. The significance of the leaf damage x plant volume interaction term in late July for the probability of raceme formation could be explained when focusing on plants

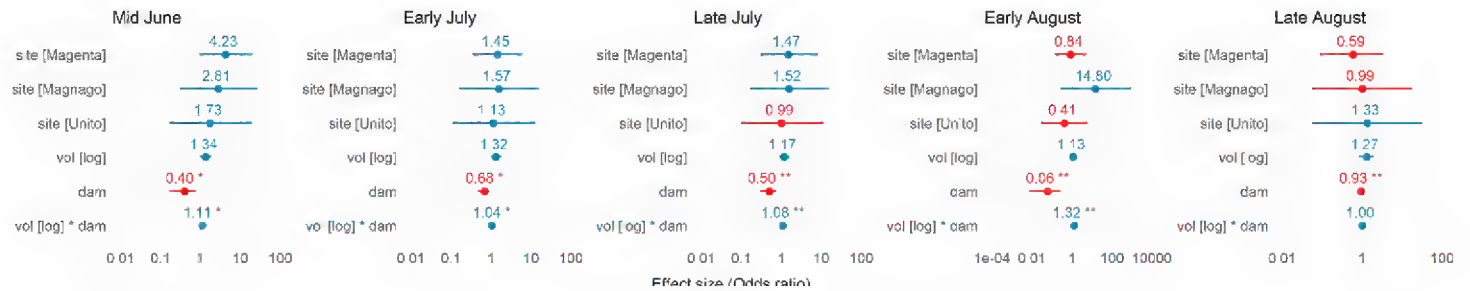

Figure 5. Effect size of explanatory variables of the models with the lowest AIC per census, explaining successful seed formation. Red dots/values <1 indicate that the effect is negative, blue dots/values >1 indicate that the effect is positive. The factor "site" with the corresponding site name in square brackets shows the effect size of site compared to Busto. Plant volume "vol" (in cm^3) is log-transformed for the analysis, leaf damage in percent is abbreviated with "dam". In models with interaction between leaf damage in percent and volume, the effect size of this factor is described as "vol[log]*dam".

with higher percent leaf damage. Plants with 1–33% leaf damage were on average smaller than plants with 34–66% leaf damage (see Figure 6A).

With regard to the models for successful seed formation, all models with the lowest AICc values calculated from mid-June to late August included percent leaf damage by *O. communa*. Of the *A. artemisiifolia* plants with more than 10% leaf damage in mid-June (19.7% of all plants), none produced seeds at the end of the season (Table 2). Likewise, only 4% of the plants with more than 10% leaf damage in early August produced seeds at the end of the season. In contrast, 14.5% and 40% of the plants with no leaf damage in mid-June and early August, respectively, produced seeds at the end of the season. Models including percent leaf damage measured in early July and late August also resulted in the respective models with the lowest AICc values for the number of seeds produced by successfully reproducing plants (Table 2). In particular, including percent leaf damage in models for successful seed production generated the lowest AICc values for all censuses from mid-June, when average leaf damage was approximately 5%, to the end of August, when almost all plants were defoliated to 80–100% (Table 2, Fig. 2).

In general, models for the successful formation of racemes and seeds that included percent leaf damage had lower AICc values than those that included *O. communa* abundance parameters (Tables 1, 2). For successful raceme formation, the effect size of sites was much higher than other explanatory factors. In contrary, the effect size of site was comparable to damage, volume or damage*volume interactions for successful seed formation (Figures 4, 5). For the plants that successfully formed seeds, we found that models including damage in early July and late August explained the number of seeds produced best, and plants with more damage produced less seeds or racemes (see Table 2). Including percent bare soil did not increase any model fits.

Interaction of *O. communa* abundance or damage with plant volume

In 18 out of 20 cases, the models including an interaction of plant volume and *O. communa* abundance or damage improved the model fit for successful seed formation, and in the two cases where *O. communa* abundance or damage improved the models

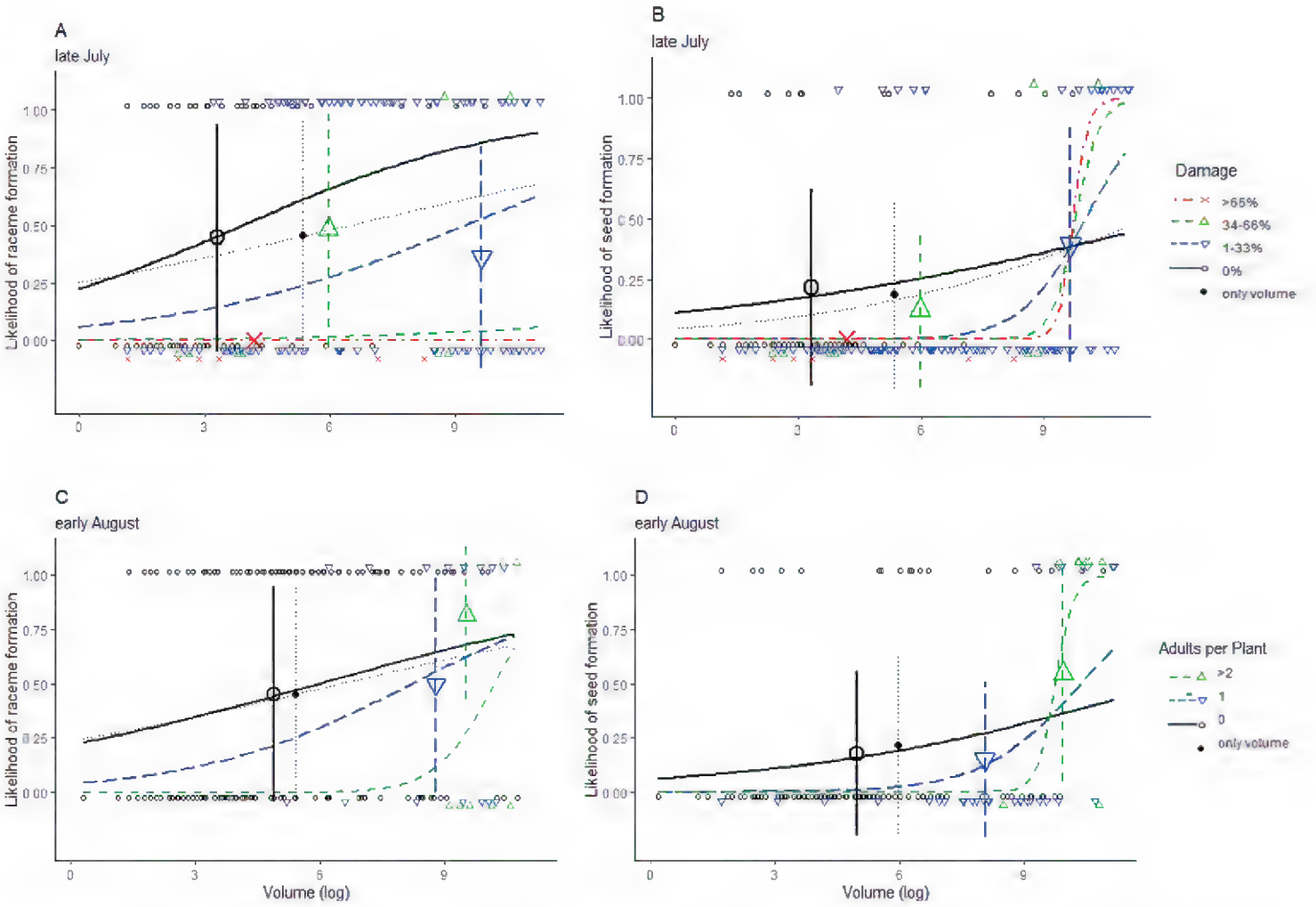

Figure 6. Likelihood of successful raceme (**A, C**) and seed formation (**B, D**) dependent on plant volume. In **A** and **B** the data are presented for four damage classes and in **C** and **D** for three *O. communa* infestation classes (0,1, or ≥2 adults per plant). The large symbols give median volume and mean probability of raceme or seed formation, respectively, together with their associated standard errors. "Only volume" shows the average values without consideration of damage or abundance classes.

for successful raceme formation, the model included an interaction of abundance or damage with plant volume. To explore the nature of these interaction terms, we displayed the interactions graphically, splitting the data into groups (by level of damage or abundance) and plotted the probability of successful raceme formation against the log of plant volume (Fig. 4). We chose late July and early August as time points for exploration, since the models with the lowest AICc values for both successful raceme and seed formation contain an interaction term with volume at these points in time, and the relationship between adult abundance and successful raceme formation was positive in late July, contrary to all other cases. Overall, leaf damage decreased and the number of adult beetles increased with plant volume, except for plants with 0% leaf damage, which were generally very small in size. The chance of raceme formation increased with plant size and decreased with percent leaf damage and the number of adults per plant.

Explained variation

The models with the lowest AICc values within one census also had the highest pseudo R-squared values. *Ophraella communa* presence and/or damage explained

Table 3. Number of plants with a certain % leaf damage producing seeds at the end of the season. Given are the number of plats within a certain damage category producing seeds / total number of plants within this damage category.

% Damage	Mid-June	Early July	Late July	Early August	Late August	Late September
0	12/83	8/59	14/66	14/35	11/18	9/14
1–10%	22/92	22/103	12/76	19/90	7/11	7/9
11–20%	0/23	4/29	6/20	2/22	0/2	4/8
21–30%	0/8	0/7	0/7	0/4	2/2	2/4
31–40%	0/4	0/3	2/3	0/4	3/3	0/3
41–50%	0/1	0/2	0/2	0/3	1/3	0/1
51–60%	0/3	0/4	0/3	0/4	2/3	0/1
61–70%	0/3	0/0	0/0	0/3	2/4	1/4
71–80%	0/1	0/1	0/1	0/1	4/5	0/6
81–90%	0/0	0/1	0/2	0/2	2/17	1/5
91–100%	0/0	0/4	0/3	0/3	1/89	5/61

the likelihood of seed formation better than the likelihood of raceme formation. Including leaf damage increased the pseudo-R^2 value of models for successful seed formation much more (max. 40%) than for raceme formation (max. 6%). In contrast, including *O. communa* abundance parameters hardly improved the pseudo-R^2 value of models of seed numbers (max. 4%) or raceme length (max. 2%). For probability of both raceme and seed formation, models had much lower AICc values and higher pseudo R-squared values from early August on. In general, *O. communa* induced leaf damage and abundance explained more variation the closer it was assessed to the flowering time.

Discussion

Our study provides evidence that the level of in-season leaf damage by *O. communa*, in combination with plant volume and site, helps to explain final seed production. Six weeks before flowering, leaf damage by *O. communa* together with plant volume is correlated to pollen production by *A. artemisiifolia* at the end of the season. Explanatory power of models improved over the season. Models including leaf damage had generally higher explanatory power than models including *O. communa* abundance parameters. For successful raceme formation, experimental sites had a much higher explanatory power than leaf damage, but for seed formation, explanatory power of leaf damage was similar to explanatory power of site, with lower variation. This offers possibilities to use in-season leaf damage for developing impact forecast models, which help informing management whether biological control is likely to successfully reduce seed production of this invasive alien plant species in a given region or year, or whether complementary management interventions should be considered to achieve long-term population decrease.

In-season *Ophraella communa* abundance and damage and plant mortality

The peak in *O. communa* abundance in early August coincides with the expected timing of the fourth and last generation in this region (Mouttet et al. 2018). We did not observe population growth of *O. communa* until late July, probably due to the relatively low densities of the different life stages and considerable variation within censuses. The lack of egg batches from September onwards is most likely due to a photoperiod-induced diapause in reproduction, as described for *O. communa* in Japan (Tanaka and Murata 2017).

While damage increased significantly in August at all sites, there was considerable variation in average leaf damage among sites (Table 2). In particular, average damage in Busto Arsizio increased to > 25% within the first three weeks of the study and was at least 25% higher than the site with the next highest average damage in early August (Magenta; Fig. 2). The high average damage in Busto Arsizio can be explained by the observations that small plants were defoliated quickly and died earlier than the extraordinarily large plants (> 2m) on this ex-arable site, while the large plants also out-shaded the smaller ones (Fig. 3). Intraspecific competition between *A. artemisiifolia* plants has been shown to have a negative effect on leaf area and aboveground biomass (Patracchini et al. 2011), and herbivory could amplify these effects by reinforcing competitive interactions (Crawley 1983).

It should be noted that our study did not cover the very first months of the growing season of *A. artemisiifolia*. In Northern Italy, gravid *O. communa* females that have overwintered start laying eggs on *A. artemisiifolia* seedlings as soon as the temperature is high enough for the beetle to fly (Bosio et al. 2014). At the study sites, first egg batches on *A. artemisiifolia* seedlings were observed from early April onwards (H. Müller-Schärer, unpublished results). Mortality of plants in spring was not covered by our experimental set-up, but is likely to further increase the impact of *O. communa* on *A. artemisiifolia* at the population level (Lommen, unpublished results).

Significant impact on target weed populations is only expected with high densities of biological control agents (Myers and Sarfraz 2017, McEvoy 2018), and the outcome of our experiment supports this notion. Defoliating *A. artemisiifolia* plants up to 90% by clipping leaves did not influence reproductive traits under laboratory conditions (Gard et al. 2013). Similarly, Lommen et al. (2017a) found that 90% defoliation of adult plants by *O. communa* in laboratory experiments did not reduce pollen production, as long as the racemes were not attacked. In our study, average leaf damage by *O. communa* only reached more than 90% in late August, which coincided with increased plant mortality.

Effect of in-season *O. communa* abundance and damage on *A. artemisiifolia* raceme and seed production

With regard to the probability of both raceme and seed formation, *O. communa* leaf damage appears to be a better explanatory variable than *O. communa* abundance, since

AICc values were lower for models including damage than those including abundance for all census dates. This could be due to the behaviour of the beetle; *Ophraella communa* adults are highly mobile (Yamanaka et al. 2007) and larvae have been observed to regularly move between plants under laboratory and outdoor conditions (Stéphanie von Bergen, personal communication). So, while leaf damage reflects the cumulative effect of the feeding activity of the beetle over time, beetle abundance represents rather a certain point in time. In weed biocontrol, impact assessments often focus on the abundance of biological control agents that are required to control a certain number of plants. For example, the impact of different life stages of *Zygogramma bicolorata* on *Parthenium hysterophorus* (Shabbir et al. 2016), or the estimation of *O. communa* impact on *A. artemisiifolia* in China (Guo et al. 2011, Chen et al. 2013) were estimated in number of adults per plant. This may be useful information when the herbivore load per plant is augmented by mass-releasing biological control agents, but it remains difficult to estimate absolute abundances of insects in field settings (Fowler and Witter 2017). Furthermore, the per capita impact may vary depending on the plant size (Biere et al. 2017), shading conditions (Muth et al. 2008), nutrient composition of the plant leaves (Zehnder and Hunter 2009), or the amount of induced defence compounds in the plants (Burghardt and Schmitz 2015). Our results suggest that for the leaf beetle *O. communa*, and probably other mobile insect herbivores, in-season damage levels may provide a more robust and easier-to-quantify variable for projecting potential impact on reproductive output of *A. artemisiifolia* at the end of the season. Similarly, early season feeding damage of the bug *Bagrada hilaris* on broccoli was also found to be a reliable and accurate variable for monitoring this pest in broccoli fields (Palumbo and Carrière 2015).

All but one model in which *O. communa* abundance explained the probability of reproductive organ formation contained an interaction with plant volume. Plant volume influences the response of the plant to abundance of herbivores or herbivore-induced leaf damage. In line with Lommen et al. (2018b), volume was positively correlated with probability of reproductive organ formation (see Figure 4, 5).

In general, we found more adults on bigger plants, probably explained by a positive effect of plant volume on adult beetle abundance, rather than a positive effect of *O. communa* abundance on plant size. Caged experiments with varying plant sizes and number of adults could shed some additional light on the potentially interacting effects of plant volume and *O. communa* impact.

The models for the likelihood of seed formation generally had higher pseudo R-squared values than the models for the likelihood of raceme formation (Tables 1, 2), indicating a larger amount of variation in the models for successful raceme formation remaining unexplained. Experimental site had a much higher effect size than damage or volume to explain chance of raceme formation, but for the chance of seed formation size effects of site and volume, damage or volume*damage interactions were comparable (see Figure 4, 5). This means that the effect of volume and leaf damage could be used to explain successful seed formation, but for successful raceme production, other factors might be more important. We observed that the mortality between late August (sampling time for racemes) and late September (sampling time for seed formation) increased in the plants that were not treated with insecticides compared to those treated in three of

the four sites (Fig. 3). We can explain why models for successful seed formation have less unexplained variation than models for raceme formation, if we assume that plant mortality is damage-dependent, but only obvious in late September. In that case, raceme formation would be more influenced by other factors that are not included in our model.

Our study provides evidence that the window of impact by *O. communa* on reproductive output of *A. artemisiifolia* is relatively narrow (see Fig. 2). Similarly, two biological control agents released to control *Clematis vitalba* in New Zealand only induced disease symptoms late in the season, resulting in no significant impact on plant growth and only minor reduction of area covered by the invasive weed (Paynter et al. 2006), and the impact of two biological control agents of *Parthenium hysterophorus* had different levels of impact between 1996 and 2000 depending on weather conditions (Dhileepan 2003). Thus, in regions or years with less favourable climatic conditions, the population peak of *O. communa* may be delayed or reduced to an extent that the impact of this biological control agent on the reproductive output of *A. artemisiifolia* is considerably impaired.

Arthropod demography is strongly influenced by climate, especially temperature, where an increase often results in quicker population growth. Since overall damage is strongly dependent on the number of generations, and as these are expected to increase with temperature in species with a multivoltine life cycles, damage is also expected to increase in a warming climate in the future (Möller et al. 2017). However, if rising temperatures leads to reduced relative humidity, climate change may also have a negative effect on population build-up of *O. communa*. Laboratory studies revealed that relative humidity of less than 50% during the warmest time of the day significantly reduced egg hatching rates of this biological control agent (Augustinus and Sun et al. 2020). Since biocontrol agent impact is dependent on high population densities (Myers and Sarfraz 2017, McEvoy 2018), both temperature and humidity should be considered for models predicting *O. communa* impact on *A. artemisiifolia* (Augustinus and Sun et al. 2020).

Implications for *A. artemisiifolia* management

Our findings that average leaf damage from mid-June onwards explained a significant amount of variation in the likelihood of seed formation indicates that *O. communa* feeding has a direct detrimental effect on female reproduction in *A. artemisiifolia*. Moreover, while the negative effect of leaf damage on the likelihood of pollen production only was significant in the census made in late July, *O. communa* exclusion experiments conducted in the same area revealed that *O. communa* reduces pollen production per unit area by 82% (Lommen et al. unpublished results). These findings are in line with an observed 80% decrease in airborne ragweed pollen counts in the Milano region since the establishment of *O. communa* (Bonini et al. 2015a, Bonini et al. 2015b), also during the year in which this study was conducted.

Hence, our findings suggest that percent leaf damage in mid-June or early July could be used as an indicator for the likelihood that *O. communa* significantly reduces

reproductive output of *A. artemisiifolia* at the end of the season (see Suppl. material 5). At three out of four sites, plants with more than 10% leaf damage in mid-June or early July had a very low likelihood of seed formation. The plants with more than 10% leaf damage in early July that produced seeds were all large plants (91–181 cm high in early July) growing at the Busto Arsizio site. At this site, where leaf damage had a less pronounced negative effect on seed and raceme formation, plants were far taller than at most other sites in Northern Italy. Apparently, plants with such an extraordinarily high volume are able to collect enough reserves to survive and produce seeds successfully before the stark increase of damage by *O. communa* in early to late August, while smaller plants cannot compensate for the damage caused by the beetle. Developing an *O. communa* damage forecasting model based on average leaf damage and plant volume in early summer may be used to support decision making regarding the scheduling of additional common ragweed management interventions. When population build-up is predicted not to be early and high enough to prevent flowering, mass releases through mass-rearing, as practiced in China (Guo et al. 2011, Chen et al. 2013), could be envisaged. Also, as *A. artemisiifolia* can form dense stands on fallow crop fields (Lehoczky et al. 2013, Ottosen et al. 2019), prospective management methods could be additional mowing, ploughing or mulching of the fields just before male flower formation. Decreasing these stands mechanically would result in less pollen production in a direct way, by removing these plants from the flowering part of the *A. artemisiifolia*, and indirectly by forcing *O. communa* to other *A. artemisiifolia* plants that are less accessible for mechanical control. Timing of mowing or herbicide applications along linear transport infrastructures (railways, roads) might also be a cost-efficient means of managing *A. artemisiifolia*, but at least three mowing interventions are needed (Lommen et al. 2018c). Experiments combining mowing regimes and beetle presence on *A. artemisiifolia* are needed to better clarify the joint effect on the control of this species.

Conclusion

This study provides evidence that the level of in-season leaf damage by *O. communa* helps to explain the impact of this biological control agent on seed and – to a lesser extent – pollen production by *A. artemisiifolia* at the end of the season. Leaf damage measured as early as mid-June partially explains, in combination with plant volume, the likelihood of reproductive output of *A. artemisiifolia* at the end of the season. For example, none of the plants with more than 10% leaf damage in mid-June formed seeds at the end of the season. It should be noted, though, that at extreme sites where *A. artemisiifolia* plants grow 2 m and taller (such as at Busto Arsizio), impact of *O. communa* may be largely explained by plant volume, rather than by average leaf damage in early summer. Our results suggest that in-season assessment of leaf damage and plant volume could be used to develop predictive models for *O. communa* impact on *A. artemisiifolia* seed production, similar to the approach used in crop pest forecasting.

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Supplementary material 1

Study sites

Authors: Benno A. Augustinus, Suzanne T.E. Lommen, Silvia Fogliatto, Francesco Vidotto, Tessa Smith, David Horvath, Maira Bonini, Rodolfo F. Gentili, Sandra Citterio, Heinz Müller-Schärer, Urs Schaffner

Data type: species data

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Link: https://doi.org/10.3897/neobiota.55.46874.suppl1

Supplementary material 2

Census dates

Authors: Benno A. Augustinus, Suzanne T.E. Lommen, Silvia Fogliatto, Francesco Vidotto, Tessa Smith, David Horvath, Maira Bonini, Rodolfo F. Gentili, Sandra Citterio, Heinz Müller-Schärer, Urs Schaffner

Data type: measurement

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Link: https://doi.org/10.3897/neobiota.55.46874.suppl2

Supplementary material 3

p-values for *O. communa*

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Data type: statistical data

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Link: https://doi.org/10.3897/neobiota.55.46874.suppl3

Supplementary material 4

Figure S1. Mean plant volume ± se of *A. artemisiifolia* plants measured during the experiment in the four experimental sites

Authors: Benno A. Augustinus, Suzanne T.E. Lommen, Silvia Fogliatto, Francesco Vidotto, Tessa Smith, David Horvath, Maira Bonini, Rodolfo F. Gentili, Sandra Citterio, Heinz Müller-Schärer, Urs Schaffner

Data type: statistical data

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Link: https://doi.org/10.3897/neobiota.55.46874.suppl4

Supplementary material 5

Figure S2

Authors: Benno A. Augustinus, Suzanne T.E. Lommen, Silvia Fogliatto, Francesco Vidotto, Tessa Smith, David Horvath, Maira Bonini, Rodolfo F. Gentili, Sandra Citterio, Heinz Müller-Schärer, Urs Schaffner

Explanation note: Likelihood of *A. artemisiifolia* seed formation dependent on *O. communa* leaf damage in early July. The different line types show the different responses between the sites.

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Link: https://doi.org/10.3897/neobiota.55.46874.suppl5

Supplementary material 6

Successful raceme formation

Authors: Benno A. Augustinus, Suzanne T.E. Lommen, Silvia Fogliatto, Francesco Vidotto, Tessa Smith, David Horvath, Maira Bonini, Rodolfo F. Gentili, Sandra Citterio, Heinz Müller-Schärer, Urs Schaffner

Explanation note: Summaries of selected glms, with successful raceme formation depending on different *Ophraella communa* abundance parameters, or leaf damage (in percent) inflicted by *O. communa*, per census.

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Link: https://doi.org/10.3897/neobiota.55.46874.suppl6

Supplementary material 7

Raceme length

Authors: Benno A. Augustinus, Suzanne T.E. Lommen, Silvia Fogliatto, Francesco Vidotto, Tessa Smith, David Horvath, Maira Bonini, Rodolfo F. Gentili, Sandra Citterio, Heinz Müller-Schärer, Urs Schaffner

Explanation note: Summaries of selected linear models, with raceme length depending on *O. communa* abundance parameters, or leaf damage (in percent) inflicted by *O. communa*.

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Link: https://doi.org/10.3897/neobiota.55.46874.suppl7

Supplementary material 8

Damage ~ abundance

Authors: Benno A. Augustinus, Suzanne T.E. Lommen, Silvia Fogliatto, Francesco Vidotto, Tessa Smith, David Horvath, Maira Bonini, Rodolfo F. Gentili, Sandra Citterio, Heinz Müller-Schärer, Urs Schaffner

Explanation note: Summaries of selected glms, with leaf damage depending on *Ophraella communa* abundance parameters.

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Link: https://doi.org/10.3897/neobiota.55.46874.suppl8

Supplementary material 9

Successful seed formation

Authors: Benno A. Augustinus, Suzanne T.E. Lommen, Silvia Fogliatto, Francesco Vidotto, Tessa Smith, David Horvath, Maira Bonini, Rodolfo F. Gentili, Sandra Citterio, Heinz Müller-Schärer, Urs Schaffner

Explanation note: Summaries of selected glm(m)s, with successful seed formation depending on *Ophraella communa* abundance parameters, or leaf damage (in percent) inflicted by *O. communa*.

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Link: https://doi.org/10.3897/neobiota.55.46874.suppl9

Supplementary material 10

Number of seeds

Authors: Benno A. Augustinus, Suzanne T.E. Lommen, Silvia Fogliatto, Francesco Vidotto, Tessa Smith, David Horvath, Maira Bonini, Rodolfo F. Gentili, Sandra Citterio, Heinz Müller-Schärer, Urs Schaffner

Explanation note: Summaries of selected lms, with number of seeds produced dependent on *Ophraella communa* abundance parameters, or leaf damage (in percent) inflicted by *O. communa*.

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